SPECIFIC AIMS

Cancer is the second leading cause of death in the United States, accounting for nearly 25% of all deaths (580,000/year); in 2015, 1.7 million new cases were diagnosed. Kinase dysregulation by oncogenic mutation, amplification, or translocation plays a critical role in many of these cancers, making targeted kinase inhibition with selective inhibitors an attractive therapeutic route. Numerous difficulties frustrate the development of kinase inhibitors with cancer-targeted selectivity profiles, and there is great need for methods that speed the development of new inhibitors for novel targets and second-line therapeutics for targets that have become therapy-resistant.

Recently, the importance of protonation state effects in kinase inhibitor affinity, selectivity, and kinetics has been recognized for the most well-studied of kinase:inhibitor interactions—the binding of Abl by imatinib (Gleevec), a blockbuster drug used to treat chronic myelogenous leukemia and gastrointestinal stromal tumors. In Abl:imatinib binding, the inhibitor and kinase both populate a mixture of protonation states, and the dominant protonation states change upon binding. Changes in pH also significantly modulate binding kinetics. **Failure to account for protonation state effects can lead to significant errors of several kcal/mol in quantitative modeling of kinase:inhibitor association, needlessly frustrating selective inhibitor design efforts or investigations of the physiological efficacy of inhibitors.** Given the prevalence of pK_as near intracellular pH in kinase inhibitors and the proximity of titratable groups (especially the conserved DFG loop) in many kinases of pharmacological interest, protonation state effects may be a widespread but poorly appreciated phenomenon.

We propose a combined computational and experimental approach to assess the prevalence of protonation state effects, dissect their origin, and overcome the limitations of current modeling techniques. Using existing tools to first identify candidate kinase:inhibitor systems for study, we use both novel computational techniques and experiments capable of observing protonation state effects to examine these systems in detail. Using new algorithms to treat protonation states dynamically, we will dissect the dominant contributions to protonation state effects and eliminate current limitations in the way protonation states are handled in computational drug discovery.

AIM 1. Broadly survey kinase:inhibitor structures for evidence of significant protonation state effects. While there is reason to expect many kinase:inhibitor binding events will exhibit protonation state effects, this has only been concretely established for the well-studied Abl:imatinib system. MCCE2 (MultiConformation Continuum Electrostatics) from the Gunner lab will be used to survey kinase:inhibitor complexes for likely protonation state effects by predicting protonation/tautomer populations for all ($>$1480) kinase:inhibitor complexes from the PDB. This approach—which is fast, but assumes a rigid backbone is maintained—will identify complexes for subsequent detailed investigation where protonation state effects have the potential to cause large (several kcal/mol) errors in quantitative predictions of binding affinity.

AIM 2. Dissect the magnitude and nature of protonation state effects in kinase:inhibitor systems using rigorous alchemical binding free energy calculations with dynamic protonation states. We will remove the assumption that protein and ligand remain fixed in a single protonation state by rigorously incorporating dynamic sampling of protonation states into quantitatively accurate explicit-solvent alchemical free energy calculations. Inspired by efficient protonation state sampling techniques from MCCE2, we will use nonequilibrium Monte Carlo techniques capable of astronomically boosting acceptance rates to incorporate dynamic protonation state sampling into our mixed MD/MC GPU-accelerated open source free energy code. We will then computationally probe the magnitude and nature of protonation state effects for candidate systems identified in Aim 1, examining the error in computed binding affinities incurred when fixed protonation states are used for protein and/or inhibitor, as well as which species (protein or ligand, which residues/functionalities) are primary contributors.

AIM 3. Experimentally assess computational findings on kinase:inhibitor systems expected to have significant protonation state effects. We will experimentally investigate kinase:inhibitor systems predicted to have significant protonation state effects in Aim 2. We focus on bacterially-expressed human kinase domains as a well-controlled model system for assessing the accuracy of computational modeling and providing insight into the magnitude of protonation state effects on ATP-competitive inhibitor binding. We will conduct four types of experimental investigations: NMR to site-specifically identify protonation state changes in inhibitors; isothermal titration calorimetry to dissect the contribution of protonation state effects to binding affinity at physiological pH; fluorescence assays to investigate the pH-dependence of binding affinities relevant to acidified intracellular environments in cancer cells; and clinically observed mutations that may modulate binding affinities via pK_a shifts.

Outlook. This project will create the technology to directly address protonation state effects in the quantitative prediction of ligand affinities—a major roadblock to widespread application of these techniques—and provide a direct assessment of the magnitude and pervasiveness of protonation state effects in kinase-inhibitor systems.